EXHIBIT C

ORIGINAL ARTICLE

The myth: in vivo degradation of polypropylene-based meshes

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Abstract

Introduction and hypothesis Polypropylene is a base polymer used in biomaterial applications, including sutures and mesh products, for the treatment of pelvic organ prolapse, stress urinary incontinence, and hernia repairs. Previous studies have dismissed the value of formulation additives employed in polypropylene, and the importance and necessity of an effective mesh explant cleaning protocol when characterizing explanted devices. However, both are critical to understanding the alleged degradation of polypropylene-based meshes.

Methods An effective, nondestructive, hydrolytic cleaning process, supplemented with light microscopy (LM), Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM) data, was used to evaluate 78 explanted Prolene meshes (with duration of implantation ranging from 0.4 to 11.7 years).

Results The cleaning process exposed clean, unoxidized, nondegraded Prolene fibers with smooth surfaces and with no visible evidence of gradient-type or ductile damage. LM showed identical translucent and sometimes clear, cracked/flaking material on both blue and clear fibers, instead of clear cracked/flaking material on the clear fibers and blue cracked/flaking material on the blue fibers. FTIR confirmed progressive protein removal and loss of protein absorption intensity after each cleaning step.

Conclusions Our effective cleaning of explanted Prolene meshes and subsequent analyses showed that they did not degrade in vivo, confirming the in vivo stability of properly formulated polypropylene. Instead, the cracked layer that some researchers have identified as degraded Prolene is an adsorbed protein–formaldehyde coating, resulting from the well-established formalin–protein fixation process, which occurs immediately upon placing an explant in formalin.

Keywords Polypropylene · Mesh · Explant analysis · Stability · Formalin fixation

Introduction

The polymer polypropylene (PP) is the basic building block of surgical mesh products used for hernia repair in addition to pelvic organ prolapse (POP) and stress urinary incontinence (SUI). In the early 1960s, Usher began using PP surgical meshes for the treatment of hernias, thus changing the approach for treating abdominal wall defects [1] and subsequently other tissue repair procedures. The use of PP for urogynecological repairs was reported for surgeries in the late 1960s, and became more popular for POP [2] and SUI with the introduction of tension-free vaginal tape (TVT) in the 1990s [3]. The American Urogynecology Society (AUGS) and the Society of Urodynamics, Female Pelvic Medicine, and Urogenital Reconstruction (SUFU) [4] have even stated that the use of PP midurethral slings is not only the recognized worldwide standard of care for the surgical treatment of SUI, but it is safe and effective, and has improved the quality of life for millions of women. They further noted that polypropylene mesh is widely studied and recognized as being safe and effective as a surgical implant.

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Over the past few years, a number of researchers have claimed that PP is not stable in vivo, but rather is oxidized and consequently experiences a loss of molecular weight, deterioration of physical properties, embrittlement, chain scission, the generation of carbonyl-containing byproducts, and in general, results in an overall loss of efficacy [5–12]. These researchers based their conclusions on the observation of a cracked layer on PP fibers, claiming that infrared spectroscopy demonstrated the presence of oxidative species and the absorption of histological dyes by pathological specimens. However, these studies failed to consider the natural adsorption of biological material when medical devices come into contact with bodily fluids [13], and the reaction of fixatives with biological materials coating the devices [14–16], thereby creating a polymerized proteinaceous layer covering the fiber (Fig. 1). They also either do not attempt to remove or do not adequately remove biological materials, which are generally fixed in formalin, from the devices. Formalin fixation may further affect chemical analysis, because it can shift and alter the intensity of absorptions in Fourier transform infrared spectroscopy (FTIR) analysis of tissue [17].

It is well documented that unstabilized PP oxidizes readily under ultraviolet (UV) light and upon exposure to high temperatures [18]. The well-known end results of PP oxidation are the formation of carbonyl compounds, molecular weight loss, and significant degradation of physical properties. However, properly formulated PP with high performance additives is stable in oxidizing media, including elevated temperatures, in vivo applications, and to a lesser extent, under UV light. For example, although Liebert et al. [19] reported the oxidation of unstabilized PP, his 1976 manuscript also established the profound stabilizing effects of antioxidants.

Liebert's work was followed by that of Williams, who reviewed polymer degradation in physiological environments [20]. He referenced Liebert, concluding that "activation energies for the degradation of high molecular weight polymers used in surgery vary from 30 kcal mol^{-1} to 80–90 kcal mol^{-1} and such reactions generally require heat, UV light, or high energy radiation, preferably in the presence of oxygen, to proceed. It seems certain from these conditions that no such degradation should occur within the confines of the human body." Williams followed with a 1992 *Clinical Materials* article, stating that "hydrophobic homochain polymers should be stable under in vivo conditions" [21].

Publications have identified a cracked surface on explanted polypropylene meshes and strongly attributed its presence to in vivo oxidation. However, the associated compositional analysis has been limited and inconclusive [5–12]. Given the limitations of previous explant studies, the purpose of the present study was to analyze the morphology and material chemistry of explanted Prolene urogynecological meshes cleaned via a novel and effective cleaning process.

Materials and methods

Explanted Prolene (Ethicon, Somerville, NJ, USA) meshes ($N = 78$) were obtained as part of medicolegal proceedings. The repository included eight different mesh designs for SUI or POP applications with implantation duration ranging from 0.4 to 11.7 years (Table 1). Twelve explants were received dry, whereas the remaining 66 explants were received in fixative. The explants were stored for an additional 0.1 to 6.4 years before cleaning and analysis. Institutional Review Board approval (Exponent, Philadelphia, PA, USA) was received for this study. Exemplar Prolene meshes (Ethicon) were also cleaned and analyzed to serve as control samples (TVT, $n = 8$; TVT Secur, $n = 3$; TVT Abbrevo, $n = 1$; Gynemesh, $n = 3$; Prolift, $n = 6$; Prolift + M, $n = 3$; Prosima, $n = 1$; and Prolene mesh, $n = 1$).

To reverse the well-known chemistry of fixative crosslinking, the meshes were cleaned using a process that exposed them to several reagents under varying conditions (Fig. 2). The cleaning process started with an initial distilled water soak; later rounds of cleaning used combinations of incubations in distilled water at an elevated temperature (70–80 °C) [15, 22], agitation using ultrasonication (with or without previous use of an orbital shaker) with sodium hypochlorite (bleach or NaOCl) [23], incubation in a proteinase K solution (enzymatic digestion) at an elevated temperature (58 °C) [14, 22], and corresponding treatment by ultrasonication in proteinase K. The meshes were rinsed to

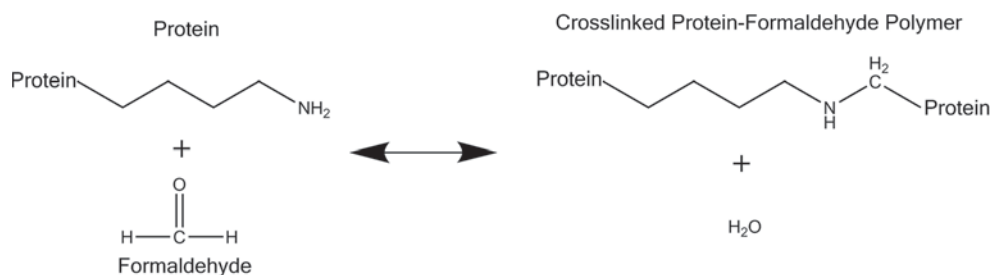**Fig. 1** Protein–formaldehyde "fixation" reaction

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Table 1 Explant summary

Patient number	Explanted Prolene mesh	Duration of implantation (years)	Duration of storage (years)	Condition explants were received in
001	TVT	7.8	0.1	Fixative
002	TVT	2.3	4.5	Fixative
003	TVT Abbrevo	0.5	4.0	Fixative
004	Prolift	7.4	1.9	Fixative
005	Prolift	2.2	4.2	Fixative
006	Prolift	4.4	2.9	Fixative
007	TVT	9.4	3.0	Dry
008	TVT	2.0	3.7	Dry
009	Prolift	4.4	3.5	Fixative
010	Prolift	4.1	3.8	Fixative
011	Prolift	3.9	3.7	Dry
012	TVT	9.3	3.6	Fixative
013	Gynemesh	9.0	0.2	Fixative
014	TVT	5.3	2.4	Fixative
015	TVT	4.2	4.2	Fixative
016	TVT	5.2	4.3	Fixative
017	Gynemesh	2.1	3.0	Dry
018	Prolift	6.4	1.2	Fixative
019	Prolift	4.8	2.0	Fixative
020	TVT	4.6	1.0	Fixative
021	Prolift	4.0–4.3[b]	3.3	Fixative
022	Prolift	7.3	1.7	Fixative
023	Prolift	4.0	3.7	Fixative
024	Prolift	4.1	2.7	Fixative
025	Prolift	5.9	2.0	Dry
026	Gynemesh	4.6	6.4	Fixative
027	Prolift	7.1	0.2	Fixative
028	TVT	5.2	0.9	Fixative
029	Prolift, TVT Secur	6.3	1.5	Fixative
030	Prolift + M	5.8	0.1	Fixative
031	Prolift + M	2.1	3.8	Dry
032	TVT	2.4	5.7	Fixative
033	Gynemesh	4.2	3.7	Fixative
034	TVT	2.6	0.1	Fixative
035	Prolift	3.0	2.0	Fixative
036	TVT	2.6	2.3	Fixative
037	Prolift	4.7	2.1	Dry
038	Gynemesh	11.7	1.2	Fixative
039	TVT	3.7–4.7[c]	0.1	Fixative
040	Prolift + M	1.9	1.7	Fixative
041	Prosima	3.1	0.7	Fixative
042	TVT	4.5	0.2	Fixative
043	TVT	6.0	0.4	Fixative
044	Prolift, TVT Secur	7.4	0.3	Fixative
045	Prolift	2.7	2.2	Dry
046	Prolift	8.0	1.0	Fixative
047	Prolift + M	1.1–2.4[d]	2.8–4.1[d]	Fixative (n = 2)
048	Prolift + M	5.8	1.2	Fixative
049	Gynemesh	3.2	1.6	Fixative
050	??[a]	?? [a]	2.9	Fixative
051	TVT	9.8	3.1	Fixative
052	Prolift	3.1	4.0	Fixative
053	Prolift, TVT Secur	5.8	3.2	Fixative
054	TVT	1.8	4.7	Dry
055	TVT	1.3	4.7	Fixative
056	TVT	2.1–8.1[d]	0.1–6.0[d]	Dry (n = 1)/fixative (n = 1)
057	TVT	6.3	3.9	Fixative
058	Prolift	4.3	4.0	Fixative
059	Prolift, TVT	2.8	4.5	Fixative
060	Prolift	4.8	2.8	Fixative
061	TVT	0.4	4.3	Fixative
062	TVT	2.5	4.3	Fixative
063	TVT	5.8	1.1	Fixative
064	Prolift	7.6	0.1	Fixative
065	Prolift	3.8	3.6	Fixative

Table 1 (continued)

Patient number	Explanted Prolene mesh	Duration of implantation (years)	Duration of storage (years)	Condition explants were received in
066	Prolift	2.3	4.2	Fixative
067	Prolene mesh	9.4	4.1	Fixative
068	Prolift	4.9	3.9	Fixative
069	TVT	6.2	2.0	Fixative
070	TVT	6.8	3.5	Fixative
071	Prolift	4.4–6.7[d]	1.0–3.3[d]	Fixative ($n = 2$)
072	Gynemesh	2.3	3.7	Fixative
073	TVT	6.9	0.2	Fixative
074	TVT	10.3	2.6	Dry
075	TVT	4.6	2.5	Dry

TVT tension-free transvaginal tape

[a] The operative report for the implantation surgery was not available for review

[b] Devices were implanted on two dates

[c] Only the year of implantation was available

[d] Devices were explanted on two dates

remove residual reagent solution from the samples after each intermediate cleaning step and before conducting materials analysis. The cleaning process shown was used for the majority of explants (85 %; 66 out of 78) analyzed; this evolved from earlier iterations of the protocol, which had used fewer steps (e.g., two cleaning cycles of NaOCl instead of

Fig. 2 Explant and exemplar cleaning protocol

Int Urogynecol J

four), additional reagents (e.g., nitric acid), and different cleaning durations.

At each intermediate cleaning step, explanted meshes and exemplar meshes underwent materials characterization and the data from the respective cleaning steps were designated as follows: Before Cleaning, After Cleaning 1, After Cleaning 2, After Cleaning 3, After Cleaning 4, and After Cleaning 5. The characterization performed at each step included light microscopy (LM), Fourier transform infrared microscopy (FTIR-Micro), and scanning electron microscopy (SEM). This technique allowed fiber examination after each step, thereby confirming loss of adhered proteins in a sequential fashion.

Light microscopy was performed using a Keyence VHX-600 digital microscopy system (Itasca, IL, USA). FTIR spectroscopy was conducted in transmission mode [24] with 64 scans at 2 cm^{-1} resolution using a Thermo Nicolet 6700 FTIR equipped with a Continuum™ Infrared Microscope (Thermo Fisher Scientific, Waltham, MA, USA). SEM imaging was performed via variable pressure mode using a Zeiss Sigma VP FEG-SEM (Carl Zeiss Microscopy, Jena, Germany) and a FEI Quanta 600 FEG (FEI, Hillsboro, OR, USA).

Results

Initial examination of the explants showed varying amounts of bulk tissue adhering to the sample after removal from the patient. Some explants were heavily covered in tissue and mesh fibers could only be observed if they protruded from the dissected edges of the specimens; other samples had less tissue adherence following explantation and mesh fibers were either clearly visible through the bulk tissue or were exposed (Fig. 3).

Light microscopy evaluation showed identical translucent and sometimes clear, cracked/flaking material on both blue and clear fibers (Fig. 4), instead of clear cracked/flaking material on the clear fibers and blue cracked/flaking material on the blue fibers. This observation was consistent across all explants containing blue fibers.

The "Before Cleaning" FTIR spectra (Fig. 5) showed spectral components of proteins as noted by the prominent 3,299- and 1,652-cm^{-1} frequencies and polypropylene via absorption frequencies at 1,452 and 1,379 cm^{-1}. The protein frequencies are due to amide N-H stretching in the 3,300-cm^{-1} region, and amide I carbonyl stretching in the region of 1,600–1,690 cm^{-1} [25]. FTIR data confirmed protein removal after each cleaning step for the explants' blue and clear Prolene fibers (Fig. 6), as demonstrated by the progressive loss of protein absorption intensity. This was confirmed via LM (described previously) and SEM (described later). The effectiveness of the cleaning process was further illustrated by overlaying the FTIR spectra of the exemplar fibers and explant fibers after the final cleaning step (Fig. 7).

In the Before Cleaning step in the Prolene explant, fibers were surrounded by bulk tissue and encased in a dry and cracked proteinaceous layer, as observed on explant surfaces via SEM (Fig. 8). In some locations, the bulk tissue had peeled from the cracked layer, exposing two surfaces that had once been a contiguous layer (see "lock and key" morphology of the tissue and cracked layer). The observable "lock and key" pattern of the formalin–protein coating demonstrated cohesive failure of the composite coating and partial adhesive failure of this same composite layer. The proteinaceous structure of the cracked layer and bulk tissue was confirmed by FTIR-Micro (described above). Cleaning progressively removed the bulk tissue and the proteinaceous shell surrounding the Prolene


Fig. 3 Overall optical microscopy images of explant samples from patients 017 (*top left*), 014 (*top right*), 022 (*bottom left*), and 021 (*bottom right*). Patient samples had varying amounts of bulk tissue and exposed mesh fibers before cleaning. Image magnification: ×20

Fig. 4 Patient 033 explant: **a** before and **b–f** after various cleaning steps. Image magnification: ×200. Had Prolene been oxidized, blue fiber flakes would be blue and clear fiber flakes would be clear; instead, identical translucent/sometimes clear, cracked and flaking material reside on both blue and clear fibers. The light microscopy images also demonstrate the successive removal of tissue using the cleaning protocol
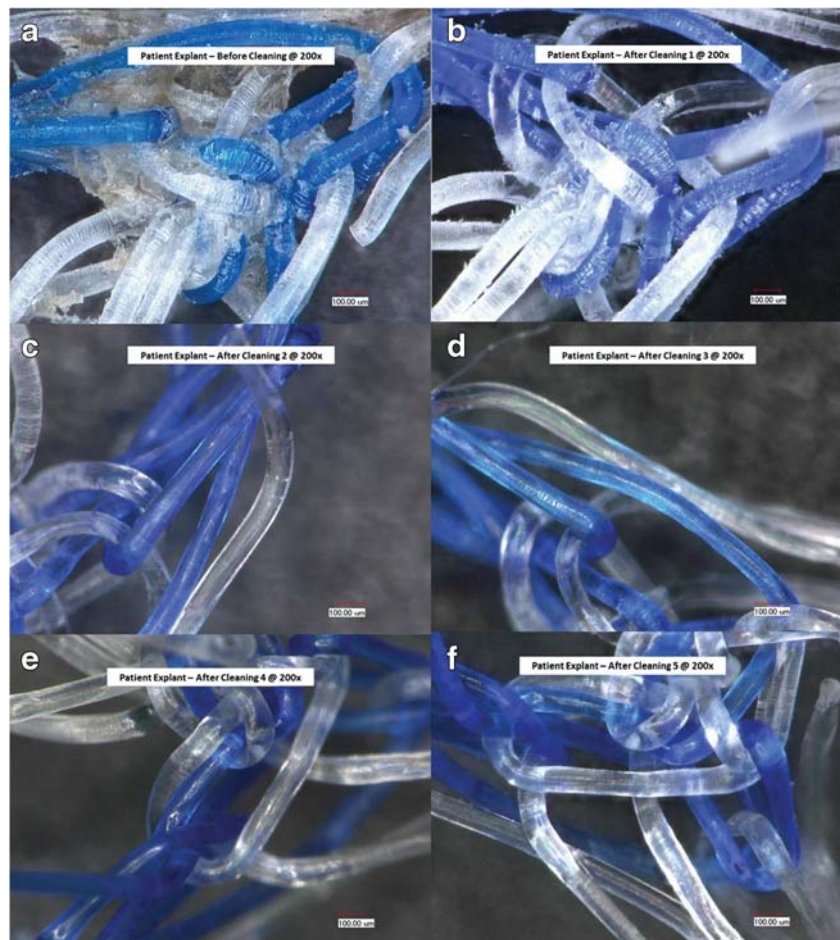
fibers. The cleaning process exposed clean, unoxidized, nondegraded Prolene fibers with smooth surfaces and with no visible evidence of gradient-type or ductile damage (Fig. 9). The cleaned fibers retained the manufacturing extrusion lines created during fiber manufacture similar to those found with the exemplar meshes (Fig. 10). Generally, the

Fig. 5 Patient 033 explant: blue fiber Fourier transform infrared spectroscopy (FTIR) before cleaning. Protein frequencies for the amide N-H stretching in the 3,300-cm^{-1} region and amide I carbonyl stretching in the region of 1,600–1,690 cm^{-1} were observed
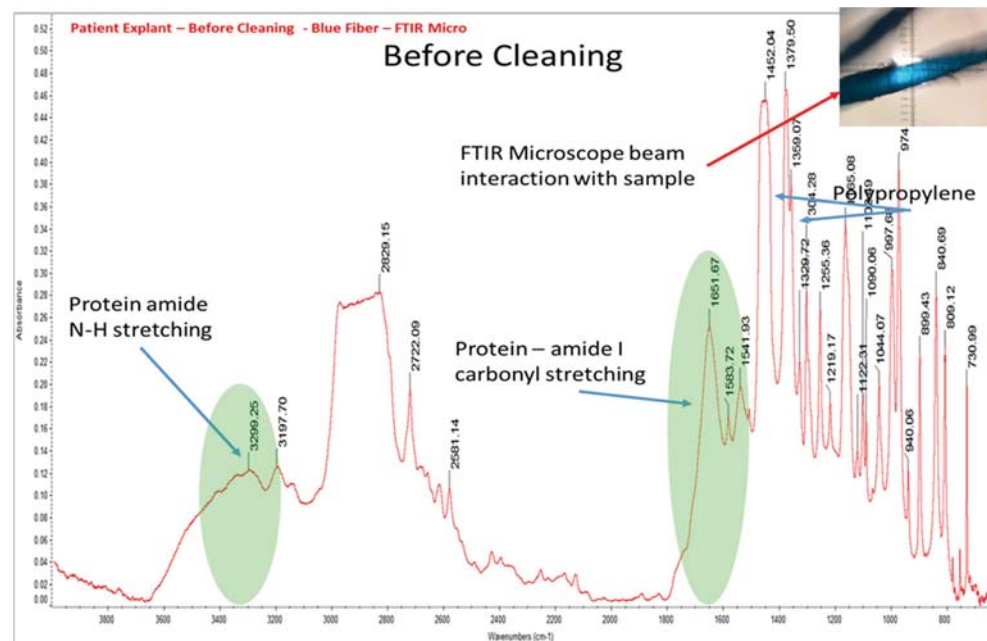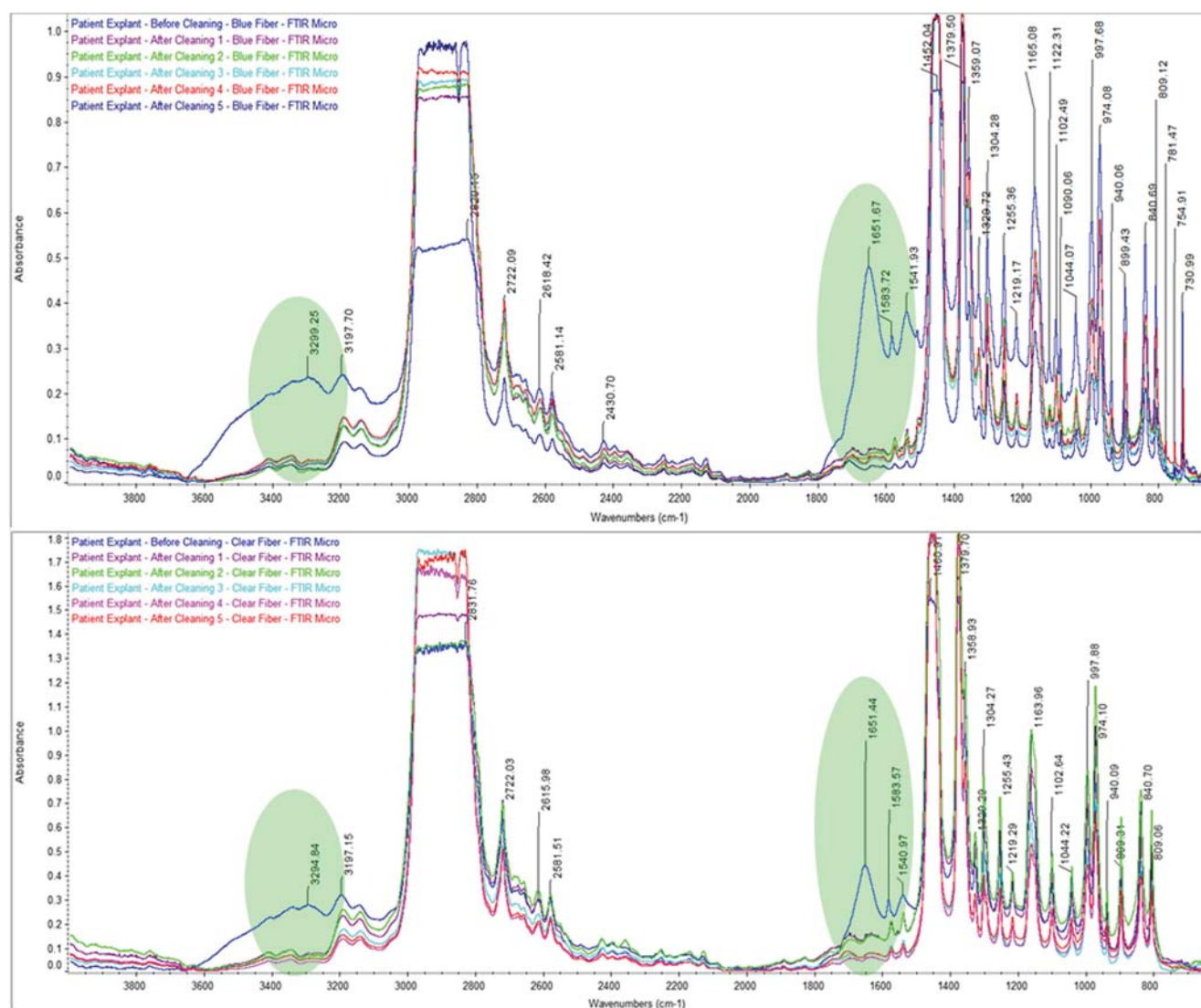
Fig. 6 FTIR of blue (*top*) and clear (*bottom*) fibers after progressive cleaning steps for Patient 033 explant, demonstrating progressive loss of proteins

tissue and biological layer appeared to be more easily removed for the explants that were received in a dry condition than those received in fixatives (Fig. 11).

Discussion

With the implementation of a novel and effective cleaning process followed by microscopy and chemical analyses, we confirmed that the explanted Prolene meshes we examined did not degrade or oxidize in vivo. FTIR absorption frequencies previously attributed by others to byproducts of oxidative degradation were observed. However, these frequencies are within the protein absorption region and are expected to be present given mesh exposure to bodily fluids, tissue ingrowth, and subsequent exposure to formalin fixatives after explantation. FTIR analyses confirmed progressive protein removal and loss of protein absorption intensity after each cleaning step.

Microscopy demonstrated progressive removal of bulk formalin fixed tissue and regions with the proteinaceous explant shell after each cleaning step. The cleaning process exposed smooth, clean, unoxidized and nondegraded fibers, with no evidence of gradient-type or ductile damage with nonuniform crack depths. Based on effective fiber cleaning and subsequent analyses, there is no evidence to support in vivo Prolene degradation.

Over the past several years, a number of researchers have claimed that PP oxidizes in vivo [5–12]. Oxidation of PP is known to result in the loss of molecular weight, deterioration of physical properties, embrittlement, chain scission, and production of carbonyl-containing byproducts [26]. In an animal study, Liebert et al. demonstrated that unprotected PP oxidizes to carbonyl compounds [19]. However, their study also found no change in the infrared spectra or tan delta of implants containing antioxidants; it was concluded that PP filaments implanted subcutaneously in hamsters degrade by an
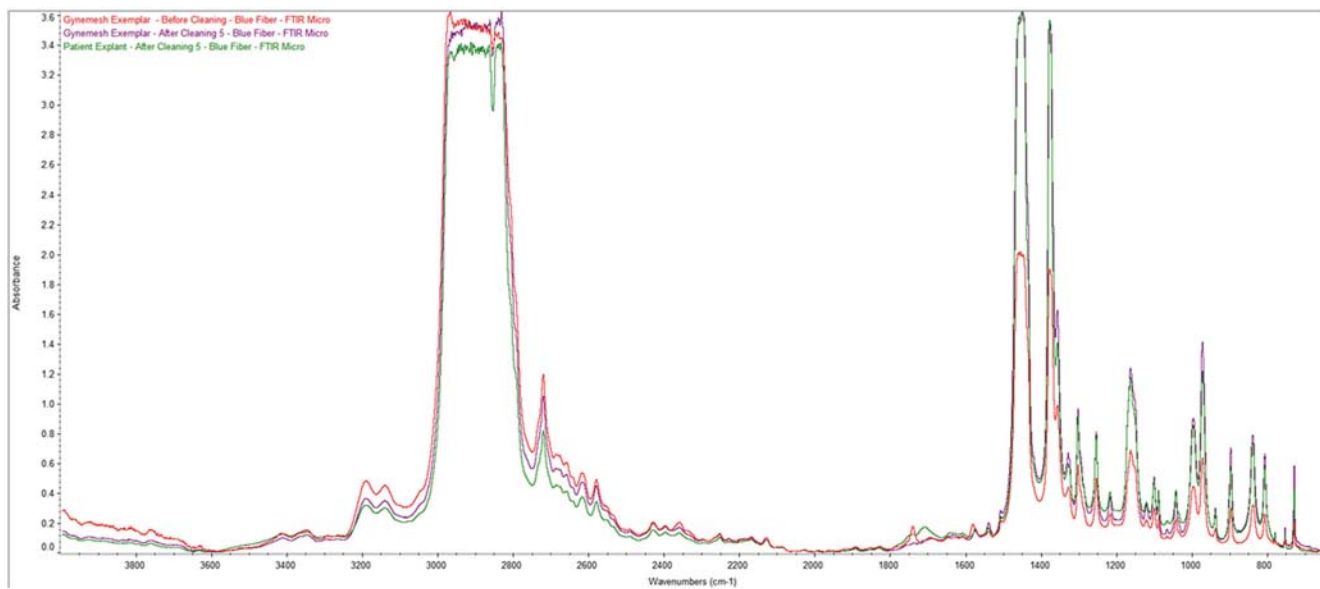
Fig. 7 Patient 033 explant and Gynemesh exemplar: FTIR of blue fibers after all the cleaning steps, illustrating overlapping spectra

oxidation process, but that the process is effectively retarded by the use of antioxidants. The authors did not observe any changes in mechanical properties or infrared spectra for any of the filaments containing antioxidants either.

Some researchers have identified cracked surfaces on explanted PP meshes and suggested that they might be a consequence of PP oxidation, although the associated compositional analysis has been limited and inconclusive [5–12]. Furthermore, these claims are based on explants whose analyses have failed to consider the effects of the fiber fixation process and the need for more effective cleaning. For example, Clavé et al. [5] has been frequently cited as supporting the notion that PP degrades in vivo. Although their manuscript title suggests that PP might not be inert, their findings clearly did not support in vivo degradation. For instance:

- DSC analyses showed that "no modification was observed in the melting temperature or heat of fusion of these samples." Their study also reported that "no difference

between DSC thermograms of pristine and degraded samples was found."
- Furthermore, they clearly indicated that "Several hypotheses concerning the degradation of the polypropylene are described below. None of these, particularly direct oxidation, could be confirmed in this study."
- In addition, their "FTIR analysis neither confirmed nor excluded oxidation of PP in the in vivo environment" nor did it "conclusively confirm that the degradation was due to oxidation."

Despite utilizing FTIR, SEM, and DSC, Clavé et al. [5] was unable to prove PP oxidation, yet continued to speak of degraded PP with no supporting data. Their observations clearly reflect artifacts from the cracked surfaces of "fixed" proteins.

Similar to Clavé et al., Costello et al. also examined formalin-fixed explanted hernia meshes [6], but used an inadequate cleaning process, which consisted only of a 2-h soak in

Fig. 8 Scanning electron microscopy micrographs from Patient 007 (*left*) and Patient 033 (*right*) before cleaning. Image magnification: ×1,000 (*left*) and ×200 (*right*)
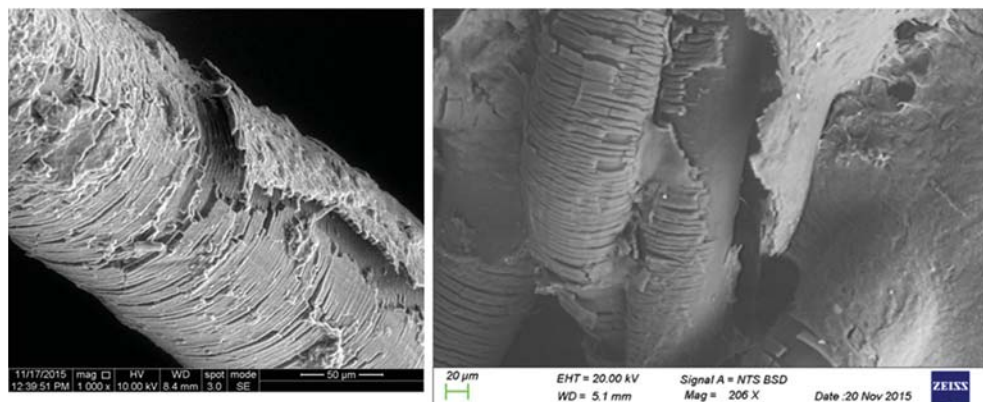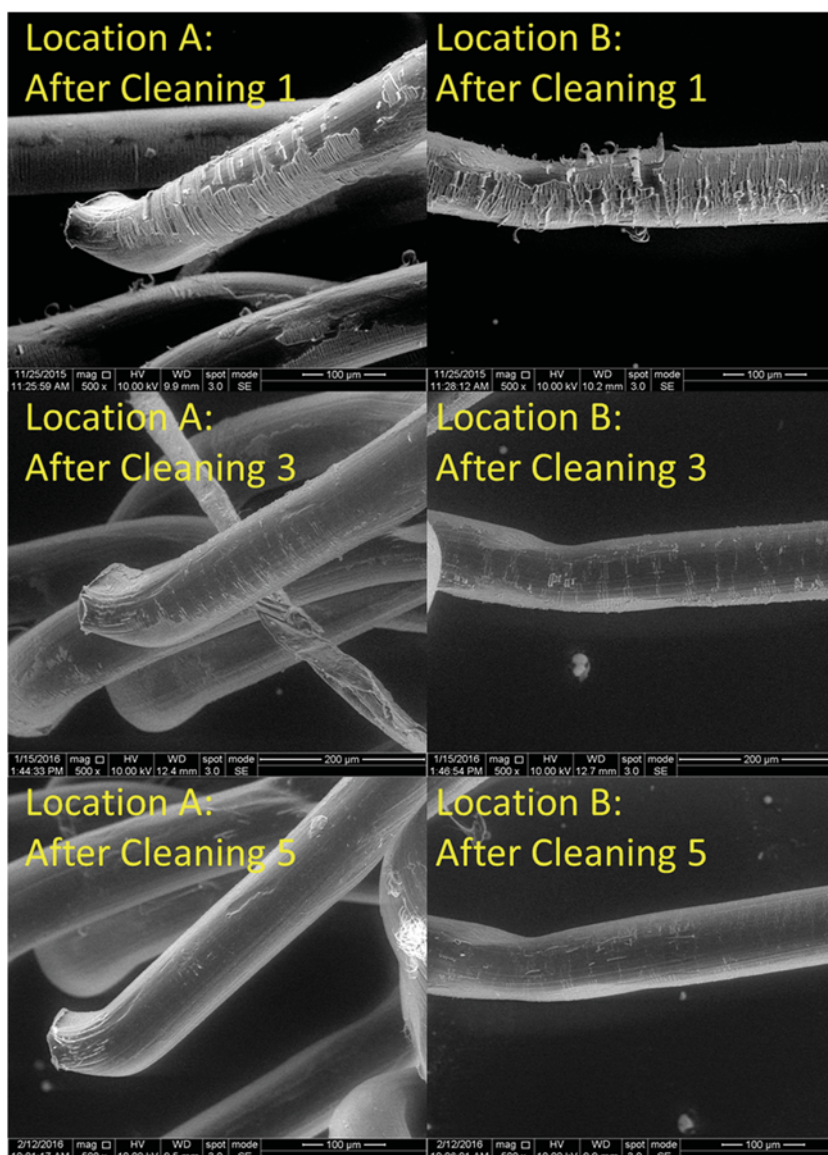
Fig. 9 Scanning electron microscopy micrographs from Patient 033 at progressive time points in the cleaning process. Images are shown for two locations: *a* (*left*) and *b* (*right*). Image magnification: ×500

sodium hypochlorite at 37 °C followed by rinsing with distilled water. Our work has shown this to be an inferior and insufficient cleaning procedure that does not completely remove "formalin-fixed" tissue. Moreover, Costello et al. did not perform FTIR analyses, which would have confirmed that the explants were not free of "fixed" proteins. Iakovlev et al. [9] did not perform any elemental or chemical analyses to support their conclusions either. Imel et al. [10] also attributed cracks on explanted PP fibers to oxidized and degraded polypropylene, using the presence of nitrogen from energy dispersive spectroscopy (EDS) analyses to distinguish between biological material and polypropylene. Yet, their own EDS analyses showed the presence of nitrogen and sodium in the cracked region; these are elements consistent with biological matter, including proteins. The findings from these previous studies are also significantly affected by the lack of any/or adequate cleaning. As discussed by Imel et al. [10], all but

one of the explanted samples were rinsed in ultrapure water and allowed to air dry. Only their sample XP-11 was treated with sodium hypochlorite solution in an attempt to remove biological tissue; however, the SEM images of XP-11 still showed the presence of biological matter. The specimens prepared by Iakovlev et al. [9] were devoid of reagents used to remove tissue and biological material from the meshes before performing histological analyses. Gross tissue and biological material were still present on the mesh fibers. As we demonstrated in our study, effective cleaning of the mesh is necessary to be able to remove the remnant biological material and examine the fiber surface that is underlying the cracked layer. This allows visualization of the transition from the cracked surface layer to the bulk fiber material to determine whether a gradient of damage exists, i.e., PP crack propagation, or whether there is a distinct interface exposing smooth, clean, unoxidized, and nondegraded fibers, i.e., a biological layer
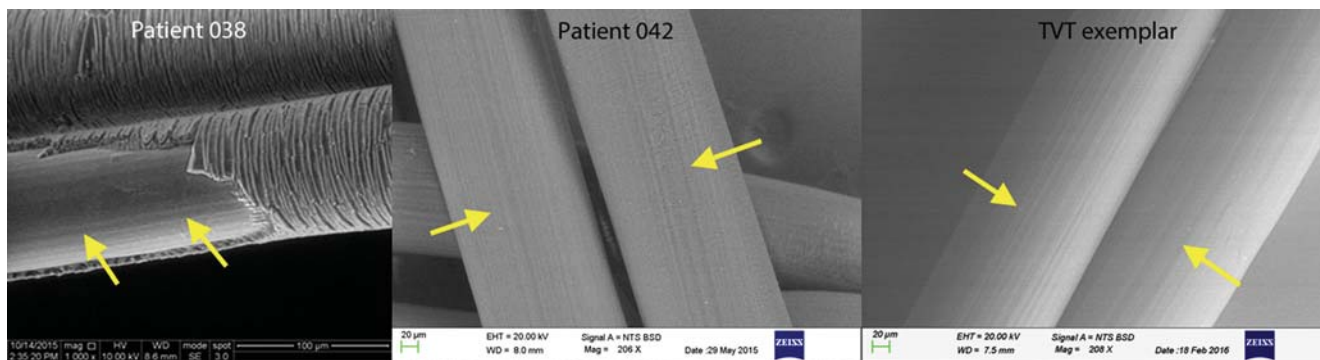
Fig. 10 The cleaning process exposed clean, unoxidized, nondegraded Prolene explant fibers with smooth surfaces and with no visible evidence of gradient-type or ductile damage. *Left* Patient 038 after cleaning sequence 1 and *middle* Patient 042 after cleaning sequence 5. The cleaned explant fibers retained the manufacturing extrusion lines created during fiber manufacture, similar to those found with the exemplar meshes that also went through the same cleaning process. *Right* tension-free transvaginal tape after cleaning sequence 5

overlaying the PP fiber. Fiber pitting was not observed either. The FTIR analysis further demonstrated that the initial carbonyl absorption of proteins disappeared as proteins were removed from the fiber surface during cleaning because of their solubility in water. On the other hand, if polypropylene had oxidized to form carbonyl bonds, the newly created hydrocarbon–carbonyl product would not have been soluble in water, would thus have remained part of the explant, and would have been detected by FTIR microspectroscopy, which was not the case.

These studies failed to consider the natural adsorption of biological material when medical devices come into contact with bodily fluids, the reaction of fixatives with biological materials adsorbed onto PP, and inadequate removal of formalin-fixed proteins. Foreign body implantation elicits immediate formation of tenaciously adsorbed and adhered "protein coating(s)" onto surfaces of implanted material(s)

[13]. Almost instantaneously following implantation, proteins interact with and adhere to the biomaterial surface through protein adsorption, creating a layer of proteinaceous coating. Even before cells reach the implant, body proteins are adsorbed onto implanted material surfaces. The adsorbed proteins form an encapsulating coating around the biomaterial (implant) surface before cells arrive and begin proliferation. Consequently, cells do not come in contact with the foreign material, but with an adsorbed and adhered protein surface.

Of great significance is that explanted PP meshes are typically stored in fixatives such as formaldehyde (formalin) or glutaraldehyde following surgical explantation. The formalin fixation process involves a chemical reaction between adsorbed biological material (proteins) and formaldehyde that creates a strong bond with the mesh fiber. The strongly adhering formalin–protein fixation product is resistant to removal/digestion from fiber(s) [14, 16]. Generally, we observed that

Fig. 11 Explants from Patient 056. *Left* sample that was received dry; *right* sample that was received in fixative after cleaning sequence 1. Light microscopy images are shown at the top (magnification: ×200; examples of the cracked layer are denoted by *arrows*; note the difference in translucency of the fibers) and scanning electron microscopy images are shown at the bottom. The cracked biological layer overlying the fibers was not as evident in the dry sample (*left*) following cleaning sequence 1 as in the fixed sample (*right*)
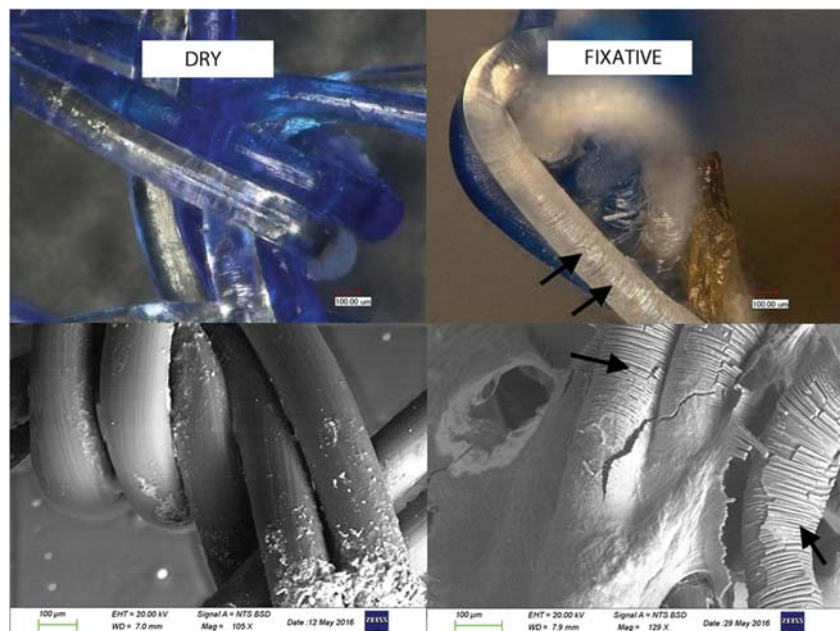
the tissue and biological layer appeared to be more easily removed for the explants that received dry than those received in fixatives (Fig. 11). However, although 12 of the explants were received dry, we were unable to verify if they had been exposed to fixative at any point before we received and analyzed them.

The chemistry of formaldehyde fixation, i.e., the chemical reaction of formaldehyde with proteins, is the chemical reaction that produces "fixed tissues." Yet this concept has been ignored or misunderstood by nonchemists when evaluating explants. Exposure to a fixative (formaldehyde) causes adsorbed proteins on mesh fibers to crosslink immediately and form a hard, brittle, protective composite layer. Fixatives are used expressly to preserve tissue and to crosslink in preparation for further study [15, 16]. A notable consequence with the use of formaldehyde, or any fixative, is the amount of distortion produced by the fixation process, which can be associated with tissue shrinkage [27]. The distortion caused by exposure to fixative can further exacerbate the effects of tissue retraction following excision, which occurs because of a loss of tension between the mesh/tissue explant and the surrounding tissue when it is excised [28, 29], thereby leading to the production of cracks in the protein coating. Previous researchers who used histological analyses to support their claims of PP degradation [13] have also failed to consider that their sample embedding processes cause tissue distortion and shrinkage [30, 31], which produces further post-excision artifacts in their analyses. Formalin fixation can also further affect chemical analysis because it can shift and alter the intensity of absorption frequencies in FTIR analysis of tissue [17]. Zhang et al. [16] notes that "In order to study the surface chemistry of explanted prostheses, it is necessary to remove all the tissue that may have grown over and within the prosthetic structure." The author also states that the degree of crosslinking may require strong chemicals and/or extreme hydrolysis to adequately clean the fiber(s).

Based on knowledge of protein adsorption onto foreign body surfaces and the "reversible" formalin fixation reaction, we developed the mild but highly effective cleaning process that we report herein. The cleaning process involves heating "formaldehyde-fixed" bulk tissue-covered explants in excess distilled water at elevated temperatures for several hours, with the inclusion of additional steps, such as treatment with sodium hypochlorite to remove excess bulk tissue, and proteinase K to assist with removal of residual and strongly adhering, formalin-fixed proteins.

Some researchers have claimed that the mesh material degrades and speculated that this might have an impact on the clinical performance of the device, through stiffening of the mesh, decreasing the mechanical properties of the mesh, playing a role in inflammation-mediated tissue damage, and increasing the need for revision surgeries due to material incompatibility [7, 9, 10]. Yet, the claimed cracking and degradation of the mesh have not produced any published reports of physical mesh breakage, nor have they been observed in our series of explants with implantation durations of up to 11.7 years. Furthermore, if there was indeed degradation of the Prolene material, the explants would be expected to exhibit a wide range of crack morphology in terms of non-uniform crack penetration at different locations for a given explant, and across explants from different patients, but this was not present in our study.

Questions and confusion about the clinical performance of transvaginal meshes had also arisen following the US Food and Drug Administration's (FDA) communication on the safety and effectiveness of transvaginal meshes for pelvic organ prolapse and mesh litigation that ensued [4, 32]. However, the professional societies have recognized that polypropylene is safe and effective as a surgical implant [4], and that polypropylene midurethral sling meshes are the standard of care for the surgical treatment of stress urinary incontinence [4]. Additionally, transvaginal meshes are not only accepted, but preferred in certain clinical situations for the treatment of pelvic organ prolapse [32]. In terms of the Prolene meshes that were examined in the present study, the polypropylene monofilaments have the same composition as Prolene sutures, which have been in use since 1969. The TVT mesh dates back to 1998, when it was cleared by the FDA [3] and has shown positive results. In a randomized controlled trial comparing TVT with colposuspension in 344 women with stress incontinence, there was a higher cure rate for the TVT patients [33]. Systematic reviews of transvaginal mesh kits have also reported a mean objective success rate of 87 % for the Prolift in treating apical prolapse [34]. Randomized controlled trials have also reported a reduction in prolapse recurrence and higher anatomical success rates for Prolift compared with other treatments, such as sacrospinous fixation [35] and anterior colporrhaphy [36], although surgical and postsurgical complication rates were higher. Studies involving the Gynemesh PS mesh have also shown improvements in the quality of life and associated improvements in prolapse symptoms, with overall anatomical success rates of 88.0 % at 1 year and 66.7 % at 5 years, in addition to a net positive effect on sexual activity following prolapse surgery, despite the use of mesh [37]. Durable prolapse repairs with 80 % anatomical success rates at 3 years have also been described following the use of Gynemesh PS [38]. Although some have claimed that mesh stiffening occurs [9], Jacquetin et al. observed moderate or severe vaginal stiffness in only 12.6 % of Gynemesh PS patients after 1 year, but this rate had not increased over time at 3 years [38].

Owing to the nature of explant analysis, our study was limited to devices that were removed during revision surgeries for various clinical reasons and we were unable to analyze implants from other patients who did not undergo revision surgery or from autopsy specimens. Because of the sample

size, statistical analysis was not performed; for example, the effects of implantation time, mesh type, storage time, patient age, and storage condition were not examined for the ease of removing the biological layer, but the results and conclusions were consistent for all our specimens. We did not quantify how much of the fiber surface was covered with the cracked biological layer for each sample after each cleaning sequence. This was due to the variability in the amount of bulk tissue that was excised with the explants during resurgery, which also appeared to affect how much biological matter was removed from location to location within a given explant. Our findings are also limited to Prolene mesh and may not necessarily apply to other polypropylene formulations, because the types and concentrations of additives, particularly stabilizers, may vary among manufacturers/suppliers.

Conclusion

Adequately formulated polypropylene, in terms of having adequate in vivo stability, along with adequate types and concentrations of additives, or in this case, Prolene, is a well-accepted biomaterial with a long history of safe and effective clinical use as a permanent implant. Our effective cleaning of explanted Prolene meshes and subsequent analyses showed that they did not degrade in vivo. Instead, the cracked layer that some researchers have identified as degraded Prolene is an adsorbed protein–formaldehyde coating, resulting from the well-established formalin–protein fixation process that occurs immediately upon placing an explant in formalin.

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